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Inhibitors of Deubiquitinating Enzymes in antiviral defence approaches

State of the art and own previous work.

Continuous optimisation of both antiviral therapies and efficient vaccination strategies is crucial to ultimately combat viral pandemics.

Our year-long reasearch reveals that three major components of the ubiquitin proteasome system (UPS) - the proteasome holoenzyme, a number of ubiquitin ligases, and a variety of deubiquitinating enzymes (DUBs) - play a crucial role in virus replication as well as in the regulation of the immunogenicity of viral proteins (Schubert et al., 2000; Schubert et al., 2000; Amit et al., 2004; Alroy et al., 2005; Setz et al., 2017; Große et al., 2022).

On the one hand, we demonstrated that certain DUB-Inhibitors (DIs) interfere with HIV-1 (Setz et al., 2017) and SARS-CoV-2 (Große et al., 2022) viral replication.

On the other hand, we and others have been elaborating the working hypothesis that the metabolic stability of an antigen inversely correlates with its entry into the UPS and the MHC-I pathway, and thus its ability to induce an efficient and specific CD8+ T cell response (Goldwich et al. 2007; Hahn et al., 2011; Setz et al. 2013; Hahn et al., 2014; Friedrich et al., 2016).

This assumption is based on the general dogma that the majority of the MHC-I epitopes is derived from so-called defective ribosomal products (DRiPs). DRiPs are *de novo* synthesised erroneous proteins, which are rapidly degraded *via* the UPS. Indeed, we could confirm that certain small drug inhibitors of DUBs can both (i) inhibit HIV-1 replication and (ii) enhance the ubiquitination, and thus the MHC-I presentation of Gag-derived epitopes (Setz et al., 2017).

Research project.

The aim of the proposed project is to elucidate the mechanism how DIs migt improve antiviral defence approaches.

First, we want to investigate the influence of DIs on the entry of viral proteins into the DRiP pathway by using biochemical methods. We also assess whether DIs enhance immune recognition of infected cells by virus specific CD8+ killer T-cells *in vitro* and *in vivo*.

Second, to explore a potential application of DUB inhibitors as T cell adjuvants, the influence of DIs on CD8+ T cell responses induced by vaccination will be studied in mouse models. By design, modern mRNA vaccines drive transient expression of protein antigens accessible to MHC class I processing machinery. CD8+ T cells are important effector cells that expand in the early protection window after prime SARS-CoV-2 spike mRNA vaccination and maintain recognition of variants of concern of SARS-CoV-2 due to epitope conservation. Since the NIH also launched clinical trials on three mRNA HIV vaccines, it also might be beneficial to co-administrate DIs together with SARS-CoV-2 and HIV-1 mRNA vaccines.